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Three-Class-Resistant Human Immunodeficiency Virus Type 1 Variant in a Drug-Naive Heterosexual Couple▽

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Combination antiretroviral treatment was initiated in a heterosexual couple newly diagnosed with human immunodeficiency virus type 1 infection. Multiple genotypic drug resistance testing following early rebound of viral load revealed that the same three-class-resistant human immunodeficiency virus type 1 strain had been present in both patients since before initiation of treatment.

CASE REPORT

Patient F was a 48-year-old female first documented to be human immunodeficiency virus type 1 (HIV-1) infected in December 2003, shortly following acute toxoplasmosis and concomitantly with thoracic herpes zoster. At that time, she had 360 CD4 cells/μl and 94,100 HIV-1 RNA copies/ml. The patient also reported persistent lymphadenopathy 1 year before, but no test for HIV-1 infection was performed on that occasion. Testing of her 42-year-old asymptomatic regular male partner (patient M) shortly afterwards also revealed HIV-1 infection, with 116 CD4 cells/μl and >100,000 HIV-1 RNA copies/ml. A first-line treatment regimen consisting of zidovudine, lamivudine, and efavirenz was immediately started in both patients. Figure 1 shows the time course of CD4 cell counts, HIV-1 RNA load, and treatment in both patients for a nearly 40-month follow-up period. The initial treatment resulted in suppression of viremia down to <50 copies/ml at week 8 in patient F and a >2-log decrease in HIV-1 RNA load in patient M but without reaching undetectable viremia. The minimum viral load for patient M was 724 HIV-1 RNA copies/ml at week 21. HIV-1 RNA rebounded to 355 and 3,020 HIV-1 RNA copies/ml at weeks 21 and 34, respectively, in patient F. At this latter time point, patient M had 12,023 HIV-1 RNA copies/ml. CD4 cell counts also decreased by 106 cells (−22%) in patient F and by 49 cells (−14%) in patient M over 3 months concomitantly with viral load rebound. The viroimmunological treatment failure observed in both patients prompted the first genotypic drug resistance test (8). Table 1 shows the resistance-related mutations (5) detected in these samples (month 9) as well as those found in the samples tested later on. At month 9, patient F and M virus populations had almost identical mutations conferring resistance to nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) (the thymidine analog mutation 1 [TAM1] profile plus the M184V mutations), nonnucleoside reverse transcriptase inhibitors (NNRTIs) (Y181C and G190A), and protease inhibitors (PIs) (L90M plus several minor mutations). The only discrepancies between patient F and M viruses were different TAM1 patterns (41L/67N/215Y versus 41L/210W/215Y) and the presence of the additional major PI resistance mutation M46I in patient F. In light of the first-line treatment failure with this associated multiclass resistance, samples stored before initiation of therapy were analyzed and found to harbor virus with the same resistance mutations as those detected in the posttreatment samples except for the absence of M184V and the presence of the "revertant" T215D in place of the T215Y resistance mutation. The same HIV-1 genotypes were confirmed at intermediate time points (months 0.8 and 1.6). Phylogenetic analysis using a random pool of HIV-1 *pol* sequences obtained in the same year from the same area confirmed the close relationship between the two viruses (Fig. 2).

Treatment was switched to a boosted PI-based regimen with a triple NRTI backbone (tenofovir, lamivudine, and stavudine) in both patients. Although cross-resistance between zidovudine and stavudine was already well known, at that time (December 2004) several algorithms predicted a higher residual activity for stavudine in the presence of a TAM1 pattern. This resulted in a sharp decrease of virus replication down to (patient F) or close to (patient M) undetectable (<50 copies/ml) levels. Both patients later underwent treatment interruptions due to personal reasons (patient M) and inability to cope with moderate toxicity (patient F). Resumption of the same (patient F) or a similar (patient M, lopinavir-ritonavir replaced by tipranavir-ritonavir) combination therapy a few months later was accompanied by a good virological response, but HIV-1 RNA remained detectable in both patients. Patient F later underwent a second treatment interruption and was finally maintained on lamivudine monotherapy up to the end of the available follow-up period. Viral suppression appeared to improve significantly at the end of the follow-up period in patient M with the switch from lamivudine to emtricitabine while maintaining stavudine, tenofovir, and boosted tipranavir. The CD4 cell count time course during the whole observation period had a significantly positive slope for patient M but not for patient F.

The only relevant changes in virus genotype during the ob-

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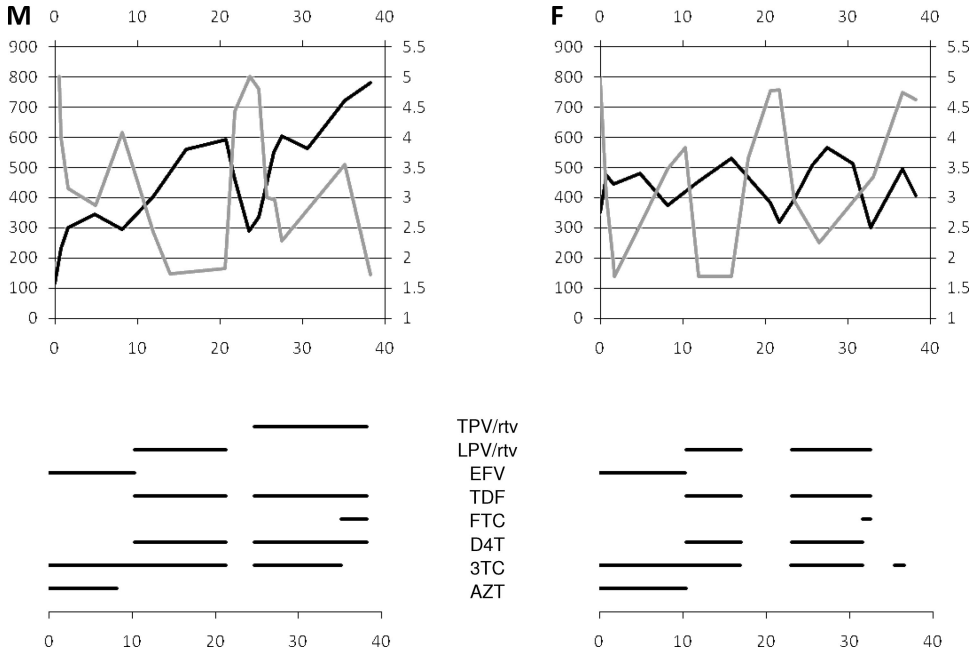

FIG. 1. Temporal course of HIV-1 RNA load (gray line, right vertical axis in log scale) and CD4 cell counts (black line, left vertical axis) for patients M and F. Horizontal axis indicates months of follow-up with respect to the first available laboratory measurement. The horizontal black lines below the graphs show the antiretroviral treatments used during the same follow-up period. 3TC, lamivudine; AZT, zidovudine; D4T, stavudine; EFV, efavirenz; FTC, emtricitabine; LPV, lopinavir; TDF, tenofovir; rtv, ritonavir (boosting dose); TPV, tipranavir.

servation period included the switch from T215D to T215Y and the selection of M184V in reverse transcriptase and the appearance of M46I in protease in the last available resistance test for patient M. However, some of the previously detected NRTI resistance mutations were not present in the last genotype obtained from the two patients (two TAMs and M184V in patient M and M184V in patient F), despite ongoing NRTI treatment. This suggests a suboptimal level of adherence to treatment in both patients at least at these later time points. However, no therapeutic drug monitoring data were available. Gradual replacement of the drug resistance T215Y with the revertant T215D reverse transcriptase mutation in patient F at the last two analyses is also in line with this hypothesis.

Development of drug resistance is a major concern for successful treatment of HIV-1 infection. The clinical impact of drug resistance has been shown to be highly relevant, particularly when susceptibility to multiple drug classes is lowered (11). HIV-1 drug-resistant variants are commonly selected under suboptimal therapy (acquired or secondary resistance) and

TABLE 1. HIV-1 protease and reverse transcriptase resistance-related mutations in the virus population harbored by patients M and F at different time points[a]

Month	Enzyme	Genotype	
		Patient M	Patient F
0.0	PR	10I 33I 60E 63P 73A 77I 90M 93L	10I 33I 46I 60E 63P 73A 77I 90M 93L
	RT	41L 181C 190A 215D	41L 67N 181C 190A 215D
0.8	PR	10I 33I 60E 63P 73A 77I 90M 93L	10I 33I 46I 60E 63P 73A 77I 90M 93L
	RT	41L 181C 190A 215D	41L 67N 181C 190A 215D
1.6	PR	10I 33I 60E 63P 73A 77I 90M 93L	10I 33I 46I 60E 63P 73A 77I 90M 93L
	RT	41L 181C 190A 215D	41L 67N 181C 190A 215D
9.0	PR	10I 33I 60E 63P 73A 77I 90M 93L	10I 33I 46I 60E 63P 73A 77I 90M 93L
	RT	41L 181C 184V 190A 210W 215Y	41L 67N 181C 184V 190A 215Y
10.7	PR	10I 33I 60E 63P 73A 77I 90M 93L	10I 33I 46I 60E 63P 73A 77I 90M 93L
	RT	41L 181C 184V 190A 210W 215Y	41L 67N 181C 184V 190A 215Y
13.9	PR	10I 13V 33I 46I 60E 63P 73A 77I 90M 93L	10I 33I 46I 60E 63P 73A 77I 90M 93L
	RT	41L 181C 190A	41L 67N 181C 184V 190A 215D/Y
25.1	PR	NA	10I 33I 46I 60E 63P 73A 77I 90M 93L
	RT	NA	41L 67N 181C 190A 215D

[a] Abbreviations: PR, protease; RT, reverse transcriptase; NA, not available.

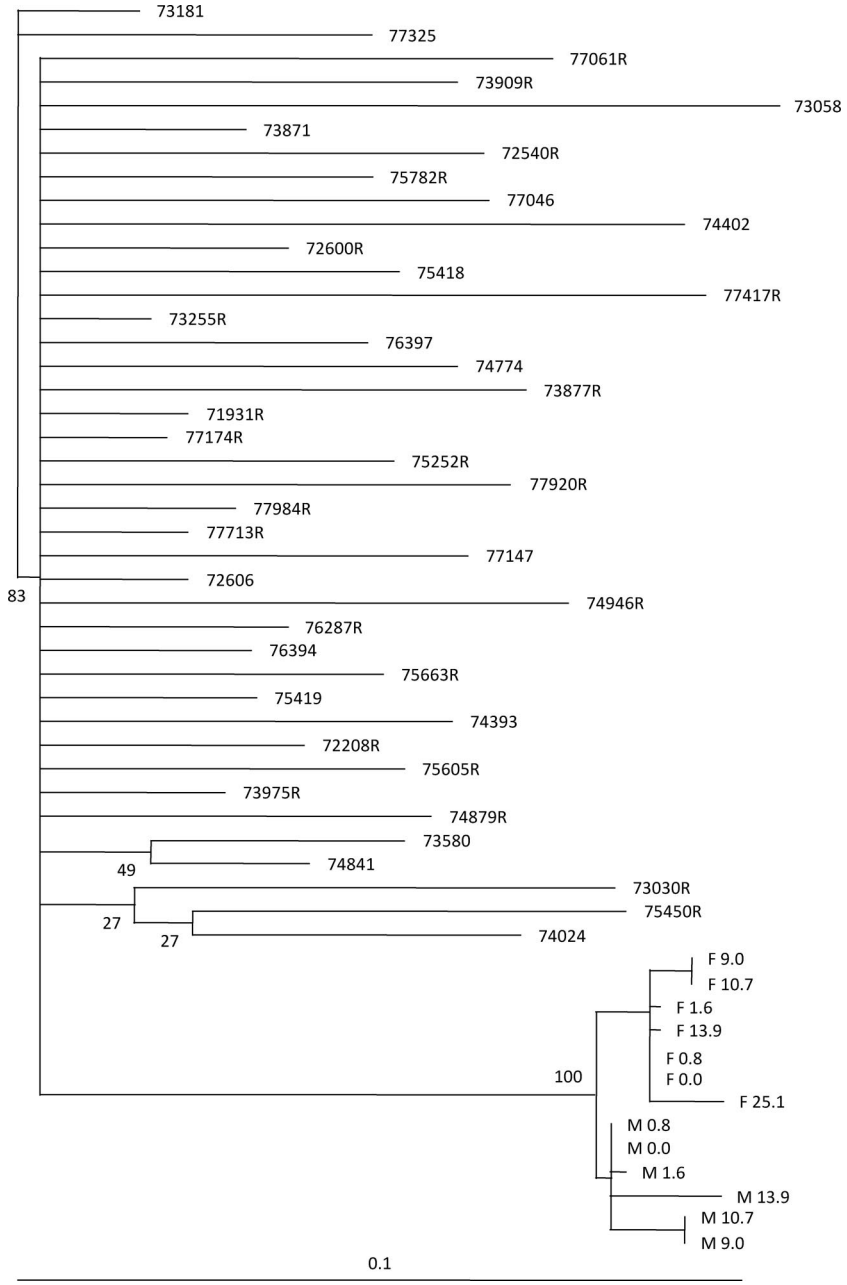

FIG. 2. Maximum likelihood phylogenetic tree showing the close relationship between patient F and M sequences. Phylogenetic analysis was conducted on a data set of 53 HIV-1 polymerase sequences aligned through ClustalX and including the seven and six sequences available from patients F and M, respectively, as well as an additional 40 sequences with drug resistance mutations obtained from the same geographic area during the same time period and randomly selected from the Siena HIV Monitoring Service database. Patient F and M sequences are indicated by F and M followed by the follow-up month number. The most appropriate model of evolution selected using ModelTest 3.7 was TVM+I+Γ, implemented into PAUP*v4b10 to estimate a maximum likelihood tree for the data set. The parameters associated with the selected model of evolution were as follows: base frequencies, A = 0.3898, C = 0.1577, G = 0.2196, and T = 0.2329; rate of substitution for A to C = 1.9457, A to G = 6.9768, A to T = 0.6498, C to G = 1.0882, C to T = 6.9768, and G to T = 1.0000; an alpha value for the gamma shape distribution of 0.7780; and a proportion of invariable sites of 0.4104. Numbers at tree nodes indicate the bootstrap values.

can be transmitted to newly infected subjects (transmitted or primary resistance). Large surveys have estimated that 10 to 25% of drug-naive infected patients harbor HIV-1 with mutations for resistance to at least one antiretroviral class, although resistance to all three major classes (NRTIs, NNRTIs, and PIs) is much less common (4). Subjects newly infected with drug-resistant virus can potentially infect other individuals, further spreading drug resistance among untreated patients (2).

Detection of closely related drug-resistant HIV-1 variants in epidemiologically linked drug-naive subjects has been occasionally documented (9, 10). This is the first report of likely onward transmission of a three-class-resistant HIV-1 from one

to another drug-naive patient. There were no laboratory or anamnestic data indicating whether transmission occurred from patient F to patient M or vice versa. The clinical presentation of patient F and the CD4 cell counts of both patients suggest that they were not acutely infected. The lower CD4 cell counts in patient M at admission to hospital may favor the hypothesis of M-to-F transmission, but both patients had probably been long infected, decreasing the confidence in this assumption. Theoretically, the possibility remains that both patients were infected by a third subject. However, both patients F and M were strictly heterosexual and had been in a relationship for more than 10 years, had never had blood transfusions, and denied use of intravenous drugs, making exposure to a common third source of infection quite unlikely. Indeed, the only risk factor declared by patients F and M was occasional heterosexual contacts with other partners before starting their relationship. While phylogenetic analysis clearly indicated a common origin for the viruses, there were subtle differences in drug resistance mutations in the first samples available from the two patients, including D67N in reverse transcriptase and M46I in protease. Diverse major histocompatibility complex-restricted recognition of viral epitopes may have driven a slightly different virus evolution in the absence of treatment in the two infected hosts (6). However, mutation M46I did appear in patient M in the last available genotype test while he was on therapy. Although M46I may have independently evolved at a late stage in patient M, its presence in the closely related patient F virus since her earliest genotypic test raises the possibility that an M46I-containing low-frequency virus population was also acquired by patient M and later contributed to the last mutational pattern detected under lopinavir-ritonavir pressure. Clonal analysis or ultrasensitive genotyping of the first patient M sample could shed light on this hypothesis.

Overall, there was not much evolution in the HIV-1 genome under therapy. While the revertant T215D mutation changed into the T215Y NRTI resistance mutation, both NNRTI and PI resistance mutations were remarkably stable during the whole treatment period. This implies that the three-class-resistant virus had acquired a constellation of compensatory mutations preserving its function at a limited cost in replicative capacity. Resistance to NNRTI is believed to impact viral fitness minimally (3). By contrast, several reports have indicated decreased replicative capacity in the context of major PI resistance mutations (1). However, compensatory mutations can rescue the original viral fitness even for highly resistant proteases (7). In line with this possibility, it is noteworthy that HIV-1 protease maintained its full complement of resistance mutations in the last available patient M genotype concomitantly with a possible decrease in adherence to treatment suggested by a lack of some of the resistance mutations previously detected in reverse transcriptase.

Notably, treatment appeared to work quite well despite the extensive resistance pattern. Even the first-line therapy, started without knowledge of genotype and actually including partially or completely ineffective drugs, yielded a significant decrease in viral load in both patients. Following the first genotypic testing, the boosted PI-based treatment lines were not completely successful primarily because of adherence issues. Nevertheless, this case highlights the potential for spreading of multiple-class-resistant HIV variants and reinforces the need for surveillance of transmitted resistance in newly infected subjects.

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